REMARKS/ARGUMENTS

Applicant confirms that the application number 09/698,106, assigned to all but the first page of the Response of April 25, 2005, should have instead been 09/206,852 and thanks the Examiner for identifying the error.

There are now 23 claims pending.

Claims 1, 21 and 22, and dependent claims thereto, have been restricted to a "leguminous" plant. Support for the amendment may be found implicitly in the specification as originally filed.

Claim 24 has been amended to specify that the plasmid vector comprises a gene for barley oxalic acid oxidase.

Claims 14 and 15 have been canceled. These claims should have been previously canceled in the Response of April 25, 2005 on the basis of an amendment made to claim 1, step (b) to specify that the contact area below the meristematic tissue of the dicot plant is a plant root suspended in a buffer. Accordingly, the subject matter of claims 14 and 15 was rendered redundant.

Claims 16, 20 and 23 have been canceled.

New dependent claims 25 to 32 have been added to define further limitations of the method of claim 22. Support for these claims may be found in the specification as originally filed.

Rejection under 35 USC §112 – Claim 23

Claim 23 has been canceled thus rendering the rejection moot thereto regarding insufficient antecedent basis for "plasmid vector".

Rejection under 35 USC §112 – Claims 1, 3, 5 to 13, 21 and 23

The Examiner has rejected claims 1, 3, 5 to 13, 21 and 23 alleging that while the specification is enabling for a plasma vector having a T-DNA region and border sequences, the specification is not enabling for any type of DNA. The Examiner refers to the paragraph bridging pages 9 and 10 of the Songstad *et al.* reference which discloses that the electrical resistance of plant tissue

affects the rate of DNA migration. On this basis, Songstad suggests that optimal migration of DNA can be achieved by minimizing the electrode distance because this will reduce the resistance of the plant and possible tissue damage caused by the strength and/or duration of the current. Accordingly, the Examiner opines that the comparatively greater electrode distance shown in Figure 1 of the instant application would likely cause tissue damage from higher electrical resistance unless T-DNA border sequences were employed to facilitate delivery of the DNA from the medium to the cells.

Claim 23 has been canceled thus rendering the rejection moot thereto.

Applicant respectfully traverses the rejection to claims 1, 3, 5 to 13 and 21.

Applicant refers to page 9, 2nd column, 2nd paragraph of the Songstad *et al.* reference which states:

> Electrophoretic-mediated DNA delivery is influenced by various physical factors associated with plant tissues...Chemical factors such as acidic pH and divalent cations affect DNA migration by reducing DNA charge...The resistance attributed to plant tissue can also affect the rate of DNA migration...Part of the resistance of plant tissue is due to the distance between the cathode and anode. [Emphasis added.]

Thus, although the Songstad *et al.* disclose that electrode distance affects DNA migration, the authors also recognize that (i) the electrode distance only partially contributes to the resistance of the plant tissue, and (ii) many experimental and physical parameters influence electrophoretic-mediated DNA delivery.

In support of further comments made below, Applicant refers to the following references of interest. A copy of reference B is enclosed with this letter and the internet source for Reference C is listed in the Appendix attached herewith:

Reference	Full Citation
(A)	U.S. Patent No. 5,371,003 (Murray *et al.*), filed on September 23, 1993 and entitled *Electrotransformation Process.*
(B)	Abstract by Sukharev S.I., Klenchin V.A., Serov S.M., Chernomordik L.V. and Chizmadzhev YuA (1992). *Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores.* Biophys J. 63(5):1320-7.
(C)	Jacobs, DF and Timmer, VR (2005). *Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function.* New Forests 30:147-166.

(A): U.S. Patent No. 5,371,003 (Murray *et al.*), filed on September 23, 1993 and entitled *Electrotransformation Process.*

Reference A discloses a method for incorporating foreign DNA into a plant host using a horizontal gel electrophoresis system. At column 6, line 34, it states that in the gel electrophoresis system employed in the examples, the electrodes are 18 cm apart.

Clearly, the electrode distance in Reference A is greater than the electrode distance shown in Fig. 3 of Songstad *et al.* and therefore, lends credence to the Songstad's comment that "[p]art of the resistance of plant tissue is due to the distance between the cathode and anode.". Accordingly, although it is suggested that DNA migration can be optimized by minimizing the electrode distance, one can deduce from Songstad *et al.* in view of Reference A that the actual distance used in any particular transformation process will depend on other factors. It can therefore be concluded that while plant resistance influences DNA migration, electrode distance is not the sole contributing factor.

Scientific Principles Regarding Resistance

Taking Ohm's Law for voltage, current, and resistance, and expressing it in terms of resistance for a given voltage and current, we have this equation:

$R = V/I$[1]

where R = resistance, V = voltage and I = current.

For any particular material, the resistance of that material can be influenced by many factors such as humidity, temperature, porosity, etc. By analogy, consider a case involving "electric shock," where electricity causes our body to experience pain or trauma. The human body has a fairly high resistance to electricity. Dry skin does not conduct electricity very well, and therefore has a high resistance, about 10 to 100 kΩ whereas moist skin is a very good electrical conductor and therefore, has a small resistance of about 1 kΩ. The table[2] below illustrates the amount of current that would be drawn if an individual made contact with an outlet in their home for 1 second having a body resistance of 10 kΩ (i.e. dry skin) and 1 kΩ (i.e. wet skin).

Electric Current Amperes	DRY SKIN Voltage for Body Resistance 10,000 Ohms	WET SKIN Voltage for Body Resistance 1,000 Ohms	Maximum Power Watts	Physiological Effect
0.001 A	10 V	1 V	0.01 W	Threshold of feeling an electric shock
0.005 A	50 V	5 V	0.25 W	Maximum current which would be harmless
0.01-0.02 A	100-200 V	10-20 V	1-4 W	Sustained muscular contraction. "Cannot let go" current.
0.050 A	500 V	50 V	25 W	Ventricular interference, pain, respiratory difficulty
0.1-0.3 A	1000-3000 V	100-300 V	100-900 W	Ventricular fibrillation. Can be fatal.
6 A	60,000 V	6,000 V	400,000 W	Sustained ventricular contraction followed by normal heart rhythm.

Clearly, these values show that for a constant current, it takes about $1/10^{th}$ the amount of voltage to achieve the same physiological effect in an individual having "wet skin" compared to having "dry skin" because of the significant drop in the skin's resistance caused by humidity. This

[1] Based on Ohm's Law, I = V/R (electric current = voltage/resistance).
[2] Table adapted from http://acept.la.asu.edu/courses/phs110/ds/appendixC.html.

further shows that the modification of one parameter compared to another parameter can have enormously different effects insofar as changing the resistance of a particular material, i.e. type of medium surrounding the material compared to electrode distance.

Studies Reveal Many Factors Affect DNA Migration in Electrophoresis

For the sake of argument only, and without being confined to any particular theory, research studies have revealed that several biological and physical parameters influence DNA migration into a plant cell membrane when an electric field is applied. Some examples of the types of biological/physical parameters are: (i) the composition of electrophoretic buffer (e.g. ionic strength, pressure, osmolarity, etc.); (ii) the cell physiology (e.g. size and shape); (iii) whether the cell incorporating the foreign DNA is a cell is destined for meiosis; (iv) the application of the electrical field insofar as its magnitude and vectorial direction; and (v) the concentration and length of the foreign DNA, to name a few.

Although all of the above factors, and others, have been studied in relation to electrotransformation and electroporation, the general consensus among researchers is that it is difficult to assess the relative contributions of all these factors on DNA delivery.

(B): Abstract by Sukharev S.I., Klenchin V.A., Serov S.M., Chernomordik L.V. and Chizmadzhev YuA (1992). ***Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores.*** **Biophys J. 63(5):1320-7.**

Applicant refers to Reference B in which the authors studied the effect of DNA interaction with membrane electropores. Based on their findings, the authors concluded that electrophoretic transfer of foreign DNA into cells is significantly enhanced by an increase in DNA concentration and the length of the DNA fragment as these factors appear to be directly correlated to an increase in the permeability of the cell membrane:

> ...The assay of electrically induced uptake of fluorescent dextrans (FDs) by cells shows that the presence of DNA in the medium during electroporation leads to a sharp increase in membrane permeability to FDs ... The permeability increases with DNA concentration ... The longer the

DNA fragment, the greater the increase in permeability. [Emphasis added.]

Still other studies have shown that when subjected to an electrical field, transient cell membrane pores are generated as a result of an increase in the dipole moment of hydrophilic phospholipid heads that move in the same direction as the applied electrical field.[3] Rapid optical measurements further support the idea that some type of rapid membrane structural rearrangement occurs, coincident with membrane conductance changes, which is consistent with pore formation.[4] On this basis, the degree of pore formation and pore diameter has been linked to transformation efficiency when a cell is subjected to an electrical field. Therefore, it has been suggested that due to structural alterations of a cell membrane induced by an electrical field, physical characteristics particular to a type of plant tissue can impart/avoid certain conformational restrictions to introducing DNA in membrane pores.

Similarly, other research studies have shown that cell-to-cell biological variability, such as cell size, causes some cells to be more sensitive to electrophoretic transformation than others. For example, cells are separated from the external medium by a closed membrane, so that interfacial polarization plays an important role by causing large changes in the transmembrane voltage, $\Delta U(t)$, by external electric field changes.[5] In the case of an isolated spherical membrane, $\Delta U(t)$ has been defined by:

$$\Delta U(t) = 1.5E(t)R_{cell} \cos(\Theta).$$

Here, R_{cell} is the cell's radius, Θ is the angle between the applied electric field, and E(t) is the site on the cell membrane at which U is measured. Generalization of this equation to nonsperical shapes also predicts a significant dependence on cell size. The above equation indicates that

[3] Biological membranes are composed of phospholipids, amphipatic molecules that have a hydrophilic head group attached to a hydrophobic tail and are able to be polarized when subjected to an electrical field. This behaviour provokes highly localized dielectric breakages in membrane structure (Neumann E., Kakorin S. and Tsoneva I. (1996), *Calcium-mediated DNA absorption to yeast cells and kinetics of cell transformation by electroporation*, Biophys. J. 71:868-877).

[4] Neumann E., Werner E., Sprafke A., Kridger K. (1992), *Electroporation phenomena: Electrooptics of plasmid DNA and of lipid bilayer vesicles.* In Jennings BR, Stoylov SP (eds): *Colloid and Molecular ElectroOptics.* Bristol: IOP Publishing, pp 197-206.

[5] Schwan HP (1989), Dielectrophoresis and rotation of cells. In Neumann E., Sowers AE, Jordan CA (eds): *Electroporation and Electrofusion in Cell Biology.* New York: Plenum, pp 3-21.

exposure to an electrical field fundamentally involves the vectorial nature of the field (magnitude and direction). This leads to many more possible "exposure" conditions at the individual cell level for the same externally applied electric field. Because of the R_{cell} dependence to transmembrane voltage, large cells generally require a smaller electrical field than small cells during membrane charging. Thus, the above equation (and its general application to nonspherical cells) indicates that variations in cell size, shape and orientation are important factors in determining the magnitude of the transmembrane voltage change for different cell membranes. The magnitude of the transmembrane voltage change, in turn, determines the amount of electrical charge applied to effect transformation. Therefore, one could expect that cells having different physical properties will not exhibit the same electrophoretical behaviour because the transmembrane voltage changes that take place upon exposure to a uniformly applied field will be different. The cell size and voltage change can all be expected to be of importance, as direct interaction of molecules with membrane pores may involve all of these properties.

(C): Jacobs, DF and Timmer, VR (2005). *Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function.* New Forests 30:147-166.

Reference C is a review article that discusses how a soil profile can dramatically affect the electrical conductivity (EC) of the soil. At page 150, it states:

> Because fertilizer nutrients are salts that conduct an electrical charge, they act to alter the EC of the soil solution. EC is the reciprocal of electrical resistance, which is measured using the equation $E = I x R$, where E is the electrical potential, I is the current, and R is the resistance...

Referring to Burchi *et al.*, this reference teaches a DNA transfer method using electrophoresis in which the cathode is placed on an axillary shoot and the anode is inserted into the soil in close contact with the roots of the plant. When read in view of Reference C, it can be concluded that the soil in which the roots of the plant are placed also influences DNA delivery because the fertilizer is composed of salts that conduct an electrical charge. Furthermore, the resistance of

the soil and of the plant have a cumulative effect on the flow of current because when different (or the same) materials are connected in series, their resistances are additive.[6]

Examiner's basis for lack of enablement rejection is speculative.

In view of the above, Applicant respectfully submits that the Examiner has merely speculated that the T-DNA border sequences may be contributing to the transformation efficiency of a plant without providing any evidentiary support to this effect. Without such support, it is improper to rely on speculative assumptions and/or interpretations regarding the claimed invention and then base a rejection on these assumptions/interpretations under U.S. patent law and practice. Thus, to merely speculate that T-DNA regions are the sole contributing factor for achieving DNA migration and to request restriction of the claimed method on this basis would deny Applicant potentially valuable protection to which it is legally entitled.

Furthermore, and to Applicant's knowledge, there is nothing in the prior art that suggests any type of plasmid vector used in electrophoresis, including a plasmid containing T-DNA regions, influences electrophoretic-mediated DNA delivery.

As a final note, under U.S. patent law, the courts have held that "it is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works."[7] Therefore, even though an inventor may not fully appreciate or realize the advantages that flow from his/her invention, or cannot give the scientific reasons for them, he/she is nevertheless entitled to its benefit as long as he/she has adequately described his/her invention so as to produce it.

Applicant therefore submits that although it is not required to give scientific reasons for why the invention works, it is the "combination" of features of the claimed invention that contribute to transformation efficiency. Further, based on the written description of the instant application, a skilled person could practice the invention without undue experimentation. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

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[6] The total resistance in a series circuit is equal to the sum of the individual resistances, i.e. RT=R1+R2+R3+... is the formula for resistance when resistances are combined in series.

[7] *Newman v. Quigg*, 877 F.2d 1575, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989).

Rejection under 35 USC §102 – Claims 16 and 20

Claims 16 and 20 have been canceled thus rendering the rejection moot thereto.

Rejection under 35 USC §103 – Claims 1, 3, 5 to 13, 16 and 20 to 24

The Examiner indicates that a typographical error exists as to the patent number of Bidney *et al.* and that the correct number is 6,166,291. Applicant is not certain as to the basis for the Examiner's comment. If, in the previous Response, this concerns the protocol of "U.S. Patent No. 5,563,005", Applicant wishes to clarify that this is the protocol practiced in Example 3 of the Bidney *et al.* patent (see column 36, lines 24 to 47) to create the soybean transgenics described therein.

The above-noted claims are further rejected as unpatentable over Burchi *et al.* in view of Bidney *et al.* In essence, the Examiner states that:

> ...it would have been obvious to use any type of vector, including the vector taught by Bidney *et al.* at column 13, lines 55-67, wherein the Ti plasmid of *A. tumefaciens* is the most widely utilized method for introducing an expression vector.

Applicant respectfully disagrees.

Burchi et al. teach a DNA transfer method into the intact meristem of adult plants grown in pots under controlled conditions using electrophoresis. The cathode is placed on the exposed meristem dome of an axillary shoot and the anode is inserted into the soil in close contact with the roots of the plant.

Bidney et al. disclose a method of producing a pathogen resistant hybrid plant using explants and a combination of wounding plant tissue by micrprojectile particle bombardment followed by co-cultivation of the explant with *Agrobacterium*. Delivery of foreign DNA into the plant host is facilitated by co-cultivation of the explant with *Agrobacterium* using the "binary" tumor-inducing (Ti) plasmid vector system.

The Examiner has failed to consider the claimed invention as a whole.

Applicant respectfully submits that the Examiner has failed to consider the claimed invention as a whole and thus, has reached an improper conclusion that the claimed method is obvious. Under U.S. patent practice, an invention is assessed based on a "combination of features" and therefore, consideration must be given to whether or not the state of the art was such as to suggest to a skilled person precisely the combination of features claimed, namely:

- contacting a meristematic tissue of a leguminous plant with a medium comprising DNA;

- suspending a root of the leguminous plant in buffer and contacting said root with a positive lead of a power source;

- contacting the medium comprising DNA with a negative lead of the power source; and

- applying a low amperage current from the power source, thereby causing the DNA to migrate from the medium to the cells of the meristematic tissue of the leguminous plant.

The fact that an individual feature (i.e. any type of vector, including the vector taught by Bidney *et al.*) or a number of features were known does not conclusively show the obviousness of a combination. The question is not whether the skilled person, with access to the entire prior art, could have made the combination according to the invention, but whether he actually would have done so in expectation of an improvement. In so doing, it is improper to focus on the obviousness of substitutions and differences between the claimed invention and the prior art rather than on the obviousness of the claimed invention as a whole relative to that prior art. In other words, it is essential to consider all elements of the claimed invention; it is impermissible to compare the prior art with what the Examiner interprets as the "gist" of the invention and to ignore the advantages, properties, utilities, and unexpected results flowing from the claimed invention as a whole.

In order to establish a prima facie case of obviousness, the prior art references(s) must teach or suggest all of the elements and limitations recited in the claims.

Applicant respectfully submits that neither Burchi *et al.* nor Bidney *et al.* teach or suggest all of the elements and limitations recited in the claimed method.

Burchi *et al.* teach a DNA transfer method into the intact meristem of adult plants grown in pots using electrophoresis in which the cathode was placed on the meristem and the anode was inserted into the soil in close contact with the roots of the plant.

Bidney *et al.* disclose a method of producing a soybean transgenic plant in which the plant was initially subjected to microprojectile bombardment, followed by co-cultivation of the plant with *Agrobacterium* species having a "binary" tumor-inducing (Ti) plasmid vector system to facilitate DNA delivery.

Neither Burchi *et al.* nor Bidney *et al.* teach or suggest all of the elements and limitations recited in the claims. For example, neither reference discloses "suspending a root of the leguminous plant in buffer and contacting said root with a positive lead of a power source" as recited in claim 1, step (b).

There is no suggestion, teaching or motivation to combine the references.

Further, there is no suggestion, teaching or motivation to combine the references on which the rejection is based. Neither of the prior art references suggests any desirability to combine the electrophoresis method, as described in Burchi *et al.*, with microparticle bombardment and *Agrobacterium* co-cultivation using the "binary" tumor-inducing (Ti) plasmid vector system, as described in Bidney *et al.* For example, one of the disadvantages of the method of transformation disclosed by Burchi *et al.* is the toxicity effect caused by the ions in the soil that accumulate near the electrode and the plant roots. A skilled person identifying this problem would not turn to the Bidney *et al.* reference in searching for a solution. Further, a skilled person could not arrive at the claimed invention if the two references were combined since they both use entirely different methods of transformation requiring entirely different set of conditions that are not transferable from one technique to the other. Burchi *et al.* use electrophoresis, whereas

Bidney *et al.* use microparticle bombardment and *Agrobacterium* co-cultivation with a plant explant. Accordingly, modification of a reference that destroys the intent, purpose or function of the invention disclosed in the reference, is not proper and the prima facie case of obviousness cannot be properly made.

No reasonable expectation of success.

In view of the above, Applicant submits that a skilled person would not have any reasonable expectation of success that the combination of Burchi *et al.* and Bidney *et al.* would work to produce beneficial results or that a person of skill in the art should be able to arrive at a claimed invention through a minimum of experimentation. Although hoping to succeed, the skilled person embarking on this project would have known that its successful conclusion depended not only on technical skill in putting into practice the sequence of precise steps of the theoretical experimental protocol, but to a large extent also on the ability to take the right decisions along the way whenever a difficult experimental situation so required. Under these circumstances, it could not be said that the skilled person would have any reasonable expectation of success since the prior art does not provide any guidance and/or direction for a person to follow in order to arrive at the claimed invention.

In summary, Applicant respectfully submits that the Examiner has not established a *prima facie* case of obviousness based on these references.

Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Examiner is respectfully urged to call the undersigned at (613) 232-2486 to discuss the claims in an effort to reach a mutual agreement with respect to claim limitations in the present application which will be effective to define the patentable subject matter if the present claims are not deemed to be adequate for this purpose.

In view of the forgoing, early favorable consideration of this application is earnestly solicited.

Respectfully submitted,

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